PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference STAN-297WO	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/US04/11533	International filing date *(day/month/year)* 14 April 2004 (14.04.2004)	Priority date *(day/month/year)* 12 April 2003 (12.04.2003)

International Patent Classification (IPC) or national classification and IPC

IPC: C12Q 1/68(2006.01);C12P 19/34(2006.01);C07H 21/02(2006.01),21/04(2006.01)
USPC: 435/6,91.2;536/23.1,23.5,24.31

Applicant

THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *(sent to the applicant and to the International Bureau)* a total of ___ sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____ , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
22 October 2004 (22.10.2004)	07 March 2006 (07.03.2006)
Name and mailing address of the IPEA/ US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer Carla Myers Telephone No. 571-272-1600

Form PCT/IPEA/409 (cover sheet)(April 2005)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US04/11533

Box No. I Basis of the report

1. With regard to the language, this report is based on:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4(a))

☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☒ the international application as originally filed/furnished

☒ the description:
pages 1-28 as originally filed/furnished
pages* NONE received by this Authority on _____
pages* NONE received by this Authority on _____

☒ the claims:
pages 29-31 as originally filed/furnished
pages* NONE as amended (together with any statement) under Article 19
pages* NONE received by this Authority on _____
pages* NONE received by this Authority on _____

☒ the drawings:
pages 1-6 as originally filed/furnished
pages* NONE received by this Authority on _____
pages* NONE received by this Authority on _____

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing (*specify*): _____
☐ any table(s) related to the sequence listing (*specify*): _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/figs _____
☐ the sequence listing (*specify*): _____
☐ any table(s) related to the sequence listing (*specify*): _____

** If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US04/11533

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 5 and 9-14

because:

☐ the said international application, or the said claim Nos. _____ relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 5 and 9-14

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US04/11533

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-4 and 6-8	YES
	Claims NONE	NO
Inventive Step (IS)	Claims NONE	YES
	Claims 1-4 and 6-8	NO
Industrial Applicability (IA)	Claims 1-4 and 6-8	YES
	Claims NONE	NO

2. Citations and Explanations (Rule 70.7)
Please See Continuation Sheet

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US04/11533

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Please See Continuation Sheet

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US04/11533

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material

   ☒ on paper

   ☒ in electronic form

   c. time of filing/furnishing

   ☒ contained in the international application as filed

   ☒ filed together with the international application in electronic form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment* on _____

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:

Claims 1, 3, 4, and 6-8 lack an inventive step under PCT Article 33(3) as being obvious over Ku (The New England Journal of Medicine, 2001). Ku teaches methods for detecting the presence of mutations in the keratin 8 gene. In particular, Ku analyzed the keratin 8 gene of patients having liver disease and normal control individuals for the presence of mutations. Ku detected the presence of the Y53H and G61C mutations in the keratin 8 gene of patients having cryptogenic liver disease. These mutations were not found in patients with other types of liver disease or in control patients. Accordingly, Ku concluded that the Y53H and G61C mutations are associated with the occurrence of cryptogenic liver disease. Ku does not specifically exemplify methods for diagnosing liver disease by detecting the Y53H or G61C mutations. However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the detection method of Ku to the diagnosis of cryptogenic liver disease since Ku teaches that the presence of the keratin 8 Y53H and G61C mutations are associated with the occurrence of cryptogenic liver disease. One would have been motivated to have done so in order to have generated a rapid, noninvasive and effective means for predicting a patient's susceptibility to cryptogenic liver disease. With respect to claim 6, Ku (page 1581) teaches amplifying the target nucleic acid prior to detecting the presence of the Y53H and G61C mutations. With respect to claim 7, Ku (page 1581) teaches using restriction enzyme digestion to detect the presence of the Y53H and G61C mutations. With respect to claim 8, Ku (pages 1581 and 1584) teaches detecting keratin 8 proteins by immunoblotting, but does not teach using antibodies that are specific for mutations in the keratin 8 protein. However, it would have been further obvious to one of ordinary skill in the art at the time the invention was made to have employed alternative methods for detecting the K8 mutations, including the use of antibodies to detect mutations, because such methods were conventional in the art at the time the invention was made and would have provided an equally effective means for detecting the Y53H and G61C mutations and diagnosing cryptogenic liver disease.

Claims 1-4, and 6-8 lack an inventive step under PCT Article 33(3) as being obvious over Ku (2002). Ku teaches methods for detecting the presence of mutations in the keratin 8 gene. In particular, Ku analyzed the keratin 8 gene of patients having liver disease and normal

Supplemental Box

control individuals for the presence of mutations. Ku detected the presence of the Y53H mutation in the keratin 8 gene of patients having cryptogenic liver disease, viral hepatitis, and biliary atresia, and the G61C mutation in patients having cryptogenic liver disease, viral hepatitis, cystic fibrosis. Additionally, a K8 G52V mutation was detected in one patient having viral hepatitis. Ku discloses that each of these mutations are associated with the occurrence of cryptogenic and noncryptogenic liver disease. Ku does not specifically exemplify methods for diagnosing liver disease by detecting the Y53H, G61C or G52V mutations. However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the detection method of Ku to the diagnosis of liver disease since Ku teaches that the presence of the K8 Y53H and G61C mutations are associated with the occurrence of liver disease. One would have been motivated to have done so in order to have generated a rapid, noninvasive and effective means for predicting a patient's susceptibility to liver disease. With respect to claim 6, Ku teaches amplifying the target nucleic acid prior to detecting the presence of the Y53H and G61C mutations. With respect to claims 7 and 8, it would have been further obvious to one of ordinary skill in the art at the time the invention was made to have employed alternative methods for detecting mutations, including allele specific probe hybridization, restriction enzyme analysis, and/or the use of antibodies to detect mutations, because these methods were conventional in the art at the time the invention was made and would have provided an equally effective means for detecting the Y53H and G61C mutations and diagnosing liver disease.

Claims 1-4, and 6-8 lack an inventive step under PCT Article 33(3) as being obvious over Ku (Molecular Biology of the Cell, 2001). Ku teaches methods for detecting the presence of mutations in the keratin 8 gene. In particular, Ku analyzed the keratin 8 gene of 323 patients having liver disease and 200 normal control individuals for the presence of mutations. Ku detected the presence of the Y53H and G61C mutation in a variety of liver diseases, including biliary atresia, hepatitis B and C, alcohol, primary biliary cirrhosis, neonatal hepatitis, congenital hepatic fibrosis, and acute fulminant hepatitis. Ku concluded that K8 mutations "are associated with variety of liver diseases and pose a risk factor for the subsequent development of liver disease." Ku does not specifically exemplify methods for diagnosing liver disease by detecting the Y53H, G61C or G52V mutations. However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have applied the detection method of Ku to the diagnosis of liver disease since Ku teaches that the presence of the K8 Y53H and G61C mutations are associated with the occurrence of liver disease. One would have been motivated to have done so in order to have generated a rapid, noninvasive and effective means for predicting a patient's susceptibility to liver disease. With respect to claim 6, Ku does not teach amplifying the nucleic acids prior to detection of the polymorphism. However, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Ku so as to have amplified the target nucleic acid prior to detecting the presence of the Y53H and G61C mutations in order to have increased the quantity of the target nucleic acid, thereby increasing the sensitivity of the detection process. With respect to claims 7 and 8, it would have been further obvious to one of ordinary skill in the art at the time the invention was made to have employed alternative methods for detecting mutations, including allele specific probe hybridization, restriction enzyme analysis, and/or the use of antibodies to detect mutations, because these methods were conventional in the art at the time the invention was made and would have provided an equally effective means for detecting the Y53H and G61C mutations and diagnosing liver disease.

---------- NEW CITATIONS ----------

NONE

VIII. The following observations on the clarity of the claims, description, and drawings or on the questions are made:

Claims 1-4 and 6-8 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because the claims are not fully supported by the description. While the description is enabling for methods of determining whether a patient has a predisposition to (i) viral hepatitis, biliary altresia, or cryptogenic cirrhosis by detecting the presence of a K8 Y53H mutation; (ii) viral hepatitis, alcohol associated liver disease, cryptogenic cirrhosis or cystic fibrosis by detecting the presence of a K8 G61C mutation; (iii) viral hepatitis or AFH by detecting the K8 R340H mutation; or (iv) viral hepatitis, alcohol associated liver disease, or biliary altresia by detecting the K8 G433S mutation, the description does not disclose the claimed invention in a manner sufficiently clear and complete to adequately enable one of skill in the art to practice methods for determining whether a patient has a predisposition to any type of liver disease by detecting any quantitative of qualitative change in the phenotype or genotype of keratin K8.

The claims are drawn to methods which determine an individual's predisposition to any type of liver disease by analyzing a sample from said individual for a quantitative or qualitative change in phenotype or genotype of keratin 8 (K8). The claims thereby include the detection of any mutation or polymorphism in the keratin 8 gene or protein or a change in the quantity of keratin 8 mRNA, DNA or protein. The claims also include the diagnosis of a wide variety of liver diseases including liver diseases associated with viral hepatitis, alcohol, cystic fibrosis, tumors, polycystic disease, parenteral nutrition-induced, multiple adenomas, and congenital hepatic fibrosis. The disclosure teaches the results of a study in which 467 liver explants from patients having liver disease and 349 healthy control blood samples were analyzed for the presence of mutations in the K8 gene. Based on this analysis, the disclosure teaches 14 mutations that were detected in the K8 gene (see Table 3). 7 of

Supplemental Box

these mutations are characterized as posing "a potential risk factor for subsequent development of liver cancer" (see footnote for Table 3). However, 3 of these mutations, namely the G52V, R453C and I-465(I) RDT(468) mutations, were found in only a single patient (i.e., 1 out of 467 patients) having liver disease. While the 3 mutations were not found in any of the 349 control blood samples, no results are provided for control liver samples. Given the fact that the 3 mutations were found in only a single patient and that the sample control population was not of an equivalent size as compared to the affected patient population and that no control liver samples were analyzed, the results obtained from this analysis would not be accepted by those of skill in the art as providing a conclusive correlation between the presence of the mutations and liver disease. With respect to the remaining 4 mutations, the disclosure teaches that these mutations were identified in specific subsets of patients having different types of liver disease. That is, the K8 Y53H mutation was found in patients having viral hepatitis, biliary altresia, or cryptogenic cirrhosis; the K8 G61C mutation was found in patients having viral hepatitis, alcohol associated liver disease or cryptogenic cirrhosis or cystic fibrosis; the K8 R340H mutation was found in patients having viral hepatitis or AFH; and the K8 G433S mutation was found in patients having viral hepatitis, alcohol associated liver disease, or biliary altresia. Accordingly, the description has enabled methods of determining whether a patient has a predisposition to (i) viral hepatitis, biliary altresia, or cryptogenic cirrhosis by detecting the presence of a K8 Y53H mutation; (ii) viral hepatitis, alcohol associated liver disease or cryptogenic cirrhosis or cystic fibrosis by detecting the presence of a K8 G61C mutation; (iii) viral hepatitis or AFH by detecting the K8 R340H mutation; or (iv) viral hepatitis, alcohol associated liver disease, or biliary altresia by detecting the K8 G433S mutation. However, the disclosure has not enabled methods for diagnosing liver disease by detecting the G52V, R453C and I-465(I) RDT(468) mutations or any other undefined mutation in the K8 gene. One can only determine whether a particular mutation is associated with liver disease by determining the sequence of the K8 gene in patients having specific types of liver disease, determining the sequence of the K8 gene in normal, healthy controls, and identifying mutations that are present at a statistically significant higher frequency in affected patients as compared to controls. While methods for sequencing genes and for performing statistical analysis are known in the art, the disclosure of such methodologies provides only an invitation to experiment. Such methodologies do not specifically lead one to the novel aspects of the claimed invention - i.e., the identity of specific mutations which are correlated with the specific types of liver disease. The results obtained with Y53H, G61C, R340H and G433S mutations cannot be extrapolated to all other mutations in the K8 gene. This finding is supported by the teachings in the disclosure in which other alterations in the K8 gene were not found to be correlated with liver disease (see pages 22-23). There is no predicable means for determining a priori which of the multitude of possible mutations in the K8 gene will or will not be correlated with a particular liver disease. The identification of an association between a mutation and liver disease can only determined through trial-by-error experimentation. Additionally, the disclosure has not established a universal association between K8 expression and the occurrence of all types of liver disease. The mechanism by which K8 contributes to or is associated with particular types of liver disease has not been disclosed. Thereby, the results obtained with specific K8 mutations and specific types of liver disease cannot be extrapolated to all other types of liver disease. Further, the disclosure does not teach an association between any type of liver disease and the presence of a change in the quantity of K8 mRNA, DNA or protein. There is no disclosure of an increase or decrease in the expression of K8 or an increase or decrease in K8 gene copy number in patients having liver disease. Moreover, no working examples have been provided in which liver disease is diagnosed based on the quantity of K8 mRNA, DNA, or protein or K8 protein activity. Accordingly, in view of the unpredictability in the art and the lack of specific guidance provided in the specification, undue experimentation would be required to practice the invention as it is broadly claimed.